the next Section). It only takes one validated recombination event[90] to rule out an equivalence between the two loci.

9.4 STARTING *FROM SCRATCH* WITH A NEW MAPPING PROJECT

9.4.1 Overview

There are two types of experimental situations in which established mapping panels may not be sufficient to the needs of an independent investigator. In the first instance, an investigator may want to pursue the mapping of a large group of cloned loci to obtain, for example, a very high resolution map for an isolated genomic region. For extended mapping projects of this and other types, it becomes both cost effective and time effective to perform an "in-house" cross for the production of a panel of samples over which the investigator has complete control.

With a second class of experimental problems, an investigator will have no choice but to perform an "in-house" cross for analysis. This will be the case in all situations where the test locus is defined only in the context of a mutant phenotype. Often, the goal of such projects will be to clone the locus of interest through knowledge of its map position. To map a mutationally defined locus, one will have to generate a special panel of samples in which segregation of the mutant and wild-type alleles can be followed phenotypically in animals prior to DNA preparation for marker locus typing. What follows in this Section is a summary of the choices that confront an investigator in the development of a mapping project *from scratch*, and the process by which an investigator should proceed through the project from start to finish.

At the outset, the investigator must make decisions concerning the form of the breeding cross itself. In particular, which parental strains will be used and what type of breeding scheme will be followed? To map a mutationally defined locus, one will obviously have to include one strain that carries the mutation. The second parental strain should be chosen based on the contrasting considerations of genetic distance (the more distant the strain, the greater the chance of uncovering polymorphisms at DNA marker loci) and the ability to generate offspring in which segregation of the mutant allele can be observed. The choice of breeding scheme is limited typically to one of two different two-generation crosses: the outcross–backcross ($F_1 \times P$, where P represents one of the original parental strains) or the outcross–intercross ($F_1 \times F_1$) illustrated in Figs 9.11 and 9.12, respectively. If the purpose of the analysis is to map loci associated with a mutant phenotype, the nature of the phenotype may limit this choice further as discussed more fully in Sections 9.4.2 and 9.4.3.

Once the strains and a breeding scheme have been chosen, one can begin to carry out the first generation cross. The number of mating pairs that should be set up need not be as large as one might think because of the expansion that will occur at the second generation. Backcrosses are usually peformed with females that are F_1 hybrids and intercrosses, by definition, are always based on F_1 hybrid females. As such, the second generation cross is likely to be highly productive

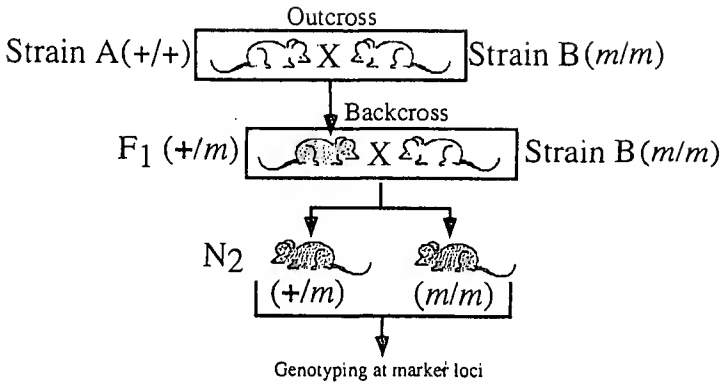

Figure 9.11 Illustration of a backcross mapping protocol. In this illustration, strain B is homozygous for a fully recessive mutation (*m*) that does not interfere with viability or fertility. All N_2 generation animals can be scored with either a mutant or a wild-type phenotype that translates directly into genotype at the *m* locus.

with larger and more frequent litters than one obtains with inbred females (see Section 4.1). Consider the goal of obtaining 1,000 offspring from several sets of an outcross–intercross or an outcross–backcross.[91] If one assumes that 90% of the second-generation mating pairs will be productive with an average of four litters with eight pups in each, one would need to set up only 35 such matings. Working backwards, to generate the 35 F_1 females and/or males required would entail only ten initial matings between the two parental strains with the assumption that 50% would be productive and these would each have three litters of five pups.

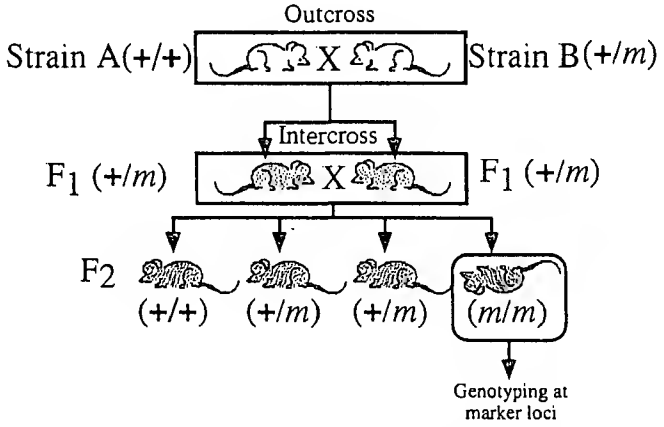

Figure 9.12 Illustration of an intercross mapping protocol. In this illustration, strain B is heterozygous for a fully recessive mutation (*m*) that causes homozygous lethality. With phenotype scoring alone, it is not possible to distinguish F_2 animals that carry +/*m* or +/+ genotypes at the *m* locus. Thus, only the mutant *m*/*m* animals can be used for linkage analysis.

An alternative backcross strategy that may sometimes be even more efficient is to set up F_1 males with inbred females of one of the two parental strains in the second generation. This approach is only effective when the backcross parent to be used is a common inbred strain such as B6. In this situation, there is no limit to the number of females that can purchased at a modest cost from various suppliers, and individual F_1 males can be rotated among multiple cages of these females. Thus, what is sacrificed in terms of hybrid vigor is made up for in terms of absolute number of crosses. As few as ten males could be rotated every 5 days among cages with two females each for a total of 120 matings in a month. One should be aware, however, that an analysis of this type will be based entirely on recombination in the male germ line, which may or may not be beneficial to the investigator according to different experimental requirements as discussed at the end of Section 9.4.4.2.

When offspring from the second-generation cross are born, one will need to analyze each for expression of the mutant phenotype. In some cases, it will be possible to use both the expression and non-expression of phenotypes as direct indicators of genotype. In other cases, it will only be possible to use phenotypic expression as an indicator of genotype in a subset of animals. This will be true for all phenotypes that are only partially penetrant as well as those that are only expressed in homozygous offspring from a second-generation intercross. In both cases, the lack of phenotypic expression in any particular animal will preclude an unambiguous determination of its genotype. When it is only possible to incorporate a subset of offspring into the ultimate genetic analysis, it will obviously be necessary to generate more offspring at the outset to achieve the same level of genetic resolution. Once "phenotyping" is accomplished, animals can be converted into DNA for incorporation into the panel that will be used for analysis of marker segregation. Optimal strategies for determining map position are discussed in Sections 9.4.4 and 9.4.5.

9.4.2 Choosing strains

9.4.2.1 For developing DNA marker maps

Upon commencing a new linkage study, an investigator will first have to decide upon the two parental mouse strains that will be used in the initial cross to generate F_1 animals. This choice will be informed by the goal of the linkage study. If the goal is simply to develop a new panel for mapping loci defined as DNA markers, there will be no a *priori* limitation on the strains that can be chosen. The most important considerations will be the degree of polymorphism that exists between the two parental strains and the ease with which they, and their offspring, can be bred to produce a large panel of second-generation animals for DNA typing.

As discussed earlier in this chapter and previous ones, the traditional inbred *M. musculus* strains show minimal levels of interstrain polymorphism. It was for this reason that the initial two-generation mapping panels were all based on interspecific crosses between a *M. musculus* strain and a *M. spretus* strain as described in Section 9.3. *M. spretus* is the most distant species from *M. musculus* that still allows the production of fertile F_1 hybrids (see Section 2.3.5). As such,